

SERUM GLYCOCONJUGATES AND CERULOPLASMIN IN CANCER OF UTERINE CERVIX

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ABSTRACT:

The present study was done to assess the levels of glycoconjugates and ceruloplasmin in sera of patients with cervical cancer. Serum hexoses, hexosamines, sialic acid and fucose are elevated in a variety of inflammatory and neoplastic conditions. All the glycoconjugates, except fucose were increased in serum of patients compared to controls. Also, hexoses and sialic acid levels were high in patients with later stages of cancer compared to patients with early stage disease ($P < 0.0001$, $P = 0.03$). Serum ceruloplasmin was increased in patients with early stage cancer (51.5mg/dl) and with late stage cancer (61mg/dl) compared to controls (38mg/dl). The elevated glycoconjugates may be the result of inflammatory reaction associated with neoplasia, as serum ceruloplasmin (an acute phase reactant) is also increased in these patients.

KEY WORDS:

Sialic acid; fucose; Ceruloplasmin; cervical cancer

INTRODUCTION :

The present study is an attempt to evaluate the changes of serum glycoconjugates in cancer of uterine cervix. The glycoconjugates in serum are hexoses, hexosamines, sialic acid and fucose. Changes in the above glycoconjugates reflect variations in glycoproteins in serum (1). The levels of the glycoconjugates are reported to be increased in squamous cell carcinoma of cervix (2). Also, serum ceruloplasmin, an acute phase reactant was measured, as an index of inflammatory changes. Ceruloplasmin levels are reported to be raised in a variety of neoplastic conditions (3, 4).

MATERIALS AND METHODS :

The study group comprised of 22 patients with histologically proven squamous cell carcinoma of the uterine cervix, who had not undergone

treatment for the cancer. Out of the 22 patients, 16 patients belonged to stages I and II (Group A) and the rest 6 patients belonged to stages III and IV (Group B). 20 age-matched females were used as controls. Protein bound hexoses were estimated by phenol-sulfuric acid method, hexosamines by the method of Elson and Morgan, modified by Winzler (5), fucose by the Dische and Shettles method (5), sialic acid by the method of Winzler (5) and ceruloplasmin by the diamine oxidase method (6). All the reagents were of analytical grade.

Statistical analyses were done between the groups by the unpaired 't' test using Statview version 3 software package. Correlation analysis was performed and was confirmed by the Fisher's r to z test.

RESULTS :

The serum glycoconjugates and ceruloplasmin levels (mean+SEM) are tabulated in Table 1. Serum hexoses, hexosamines, sialic acid and ceruloplasmin were significantly increased

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in group A and group B patients compared to controls. Also, the levels of hexoses and sialic acid were higher in group B patients compared to group A ($P < 0.0001$, $P = 0.03$). The mean hexosamine and ceruloplasmin levels were not significantly different between group A and group B patients.

Serum fucose levels were not significantly increased in group A and group B patients with cervical cancer compared to controls. Also, the fucose levels in group B patients were not significantly increased compared to group A.

A significant correlation was present between hexoses and ceruloplasmin ($n = 42$, $r = 0.602$, $P < 0.0001$), sialic acid and ceruloplasmin ($n = 42$, $r = 0.639$, $P < 0.0001$) and hexoses and sialic acid ($n = 42$, $r = 0.579$, $P < 0.0001$) as shown in Figure 1.

The sensitivity and specificity of the serum parameters are tabulated in Table 2.

DISCUSSION :

Glycoconjugates are increased in serum in a variety of inflammatory and neoplastic conditions (2, 7). The rise in the above glycoconjugates are neither tumor specific nor organ specific. In our study, there was an increase in all the glycoconjugates, except fucose, even in the early stages of cancer. The levels of hexoses and sialic acid were higher in the later stages of cancer compared to the early stages. Similar findings were also reported in cervical cancer (2, 8), in head and neck malignancy (9) and in ovarian cancer (10). However, Patel *et al* have reported limited correlation with staging in cervical cancer (11).

A rise in serum fucose levels was observed in cervical cancer (2, 12, 13). Bandlish *et al* found an insignificant elevation of fucose in localized tumors (12). We have not observed an increase in serum fucose levels in cervical cancer patients.

Similar findings were also observed by Rao *et al* in oral cancer wherein serum fucose levels did not correspond to the stage of the disease (14). Also, comparison of serum total, free and bound fucose levels between patients with colorectal cancer and controls has showed no statistically significant difference. However, when the total and bound fucose were normalized to total protein, there was an increase in both the parameters. But, the values of total fucose only were statistically significant (15).

A rise in serum ceruloplasmin was observed in cervical cancer (16, 17, 18). Arumanayagam *et al* have reported higher levels of ceruloplasmin in later stages of cancer compared to early stages (17). However, we have not observed a significant increase in serum ceruloplasmin in advanced cervical cancer compared to that in stage I and II disease. Hansen *et al* have reported a correlation between acute phase reactant levels and tumor size and burden. They have also suggested that the rise in acute phase reactants was because of non-specific inflammation in the tissues surrounding the tumor (19). There is an increase in sialic acid rich acute phase proteins in inflammation (20), which is evident in our study as shown in Figure 1.

We found that the serum glycoconjugates and ceruloplasmin are raised even in the early stages of cervical cancer. Moreover, the serum glycoconjugates, namely, hexoses and sialic acid are much higher in advanced cancer compared to early stage disease. Also, correlation between hexoses and sialic acid indicate that they probably reflect changes in similar glycoproteins. Therefore, these two glycoconjugates would be better serum parameters in evaluation of the severity of the disease. However, interpretation should be done with caution, as there is a possibility of increase in these markers due to nonspecific inflammation associated with cancer.

TABLE 1

SERUM HEXOSES, HEXOSAMINES, SIALIC ACID, FUCOSE AND CERULOPLASMIN LEVELS (MEAN+SEM) IN CONTROLS AND PATIENTS WITH CERVICAL CANCER.

Parameters	Controls (n=20)	Group A (n=16)	Group B (n=6)
Hexoses (mg/dl)	110.5±2.9	125.5±2.5*	158.7±2.2**
Hexosamines (mg/ dl)	122.3±4.9	171.3±14.1*	201±13.1**
Sialic acid (mg/dl)	82.8±2.2	99.5±5.6*	121±5.2**
Fucose (mg/dl)	14.5±0.7	15.2±0.7	16.5±1.1
Ceruloplasmin (mg/dl)	38±1.4	51.5±3.5*	61±4.8**

Groups A and B are compared to controls.

*P=<0.001

**P=<0.0001

Group A: Patients with stage I and II cervical cancer

Group B: Patients with stage III and IV cervical cancer

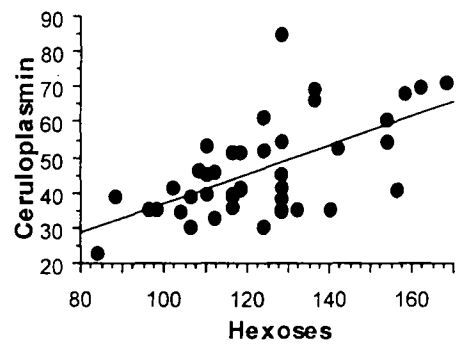
Table 2

Sensitivity and specificity of serum hexoses, hexosamines, sialic acid, fucose and ceruloplasmin in patients with cervical cancer.

Serum parameter	Sensitivity (%)	Specificity (%)
Hexoses	77	80
Hexosamines	72	100
Sialic acid	72	75
Fucose	68	85
Ceruloplasmin	72	90

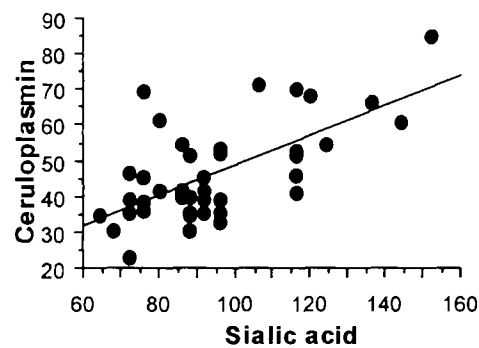
Figure 1. Bivariate scattergram showing correlation between :

A - Hexoses and ceruloplasmin



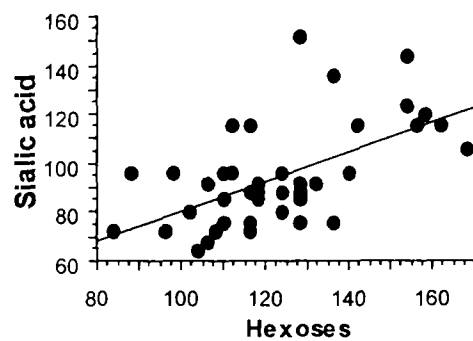
$$\text{Ceruloplasmin} = -4.814 \pm 0.416 * \text{Hexoses} ; \gamma^2 = 0.363$$

B - Sialic acid and ceruloplasmin



$$\text{Ceruloplasmin} = 6.681 \pm 0.42 * \text{Sialic acid} ; \gamma^2 = 0.409$$

C - Sialic acid and hexoses



$$\text{Sialic acid} = 19.704 \pm 0.609 * \text{Hexoses} ; \gamma^2 = 0.335$$

REFERENCES :

1. Henry R. J., Cannon, D. C. and Winkelman JW. (1974); Clinical Chemistry: Principles and Techniques Harper and Row publishers Inc. pg 461
2. Bhuvarahamurthy, V., Balasubramanian, N., Vijaykumar, S. and Govindasamy, S. (1995) Effect of radiation on serum glycoproteins and glycosidases in patients with cervical carcinoma. *Int. J. Gynaecol Obstet* 48 49-54
3. Walker, C. and Gray, BN. (1986) Acute phase reactant proteins and carcinoembryonic antigen in cancer of colon and rectum. *Cancer* 52, 150-154
4. Krishnamurthy, S. and Jaya, S. (1986) Serum tocopherol, lipo-peroxides, ceruloplasmin and red cell glutathione and antioxidant enzymes in patients with oral cancer. *Indian J. Cancer* 23, 36-42
5. Winzler, RJ. Determination of serum glycoproteins. *Methods of Biochemical Analysis*; Vol 2; Ed. Glick D; pg. 279-311
6. Ravin, HA. (1961) An improved colorimetric enzymatic assay of ceruloplasmin. *J. Lab. Clin. Med.* 58,161-8
7. Erbil, K. M., Jones J. D. and Klee, GG. (1985) Use and limitations of serum total and lipid bound sialic acid concentration as markers of colorectal cancer. *Cancer* 55, 404-409
8. Balasubramanian, N., Subramanian S., Sekar, N., Bhuvarahamurthy, V. and Govindasamy, S. (1994) Diagnostic and prognostic role of plasma and urinary sialic acid in human carcinoma of uterine cervix. *Biochem. Mol. Biol. Int.* 33, 617-23
9. Bathi, R. J., Nandimath, K., Kannan, N. and Shetty, P. (2001) Evaluation of glycoproteins as prognosticators in head and neck malignancy. *Indian J Dent Res.* 12: 93-99
10. Berbee, H., Paszkowska, A., Siwek, B., Gradziel, K. and Cybulski, M. (1999) Total serum sialic acid concentration as a supporting marker of malignancy in ovarian neoplasia. *Eur. J. Gynaecol, Oncol*, 20, 389-392
11. Patel, P. S., Rawal, G. N. and Balar D. B. (1993) Importance of serum sialic acid and lactate dehydrogenase in diagnosis and treatment monitoring of cervical cancer patients. *Gynaecol. Oncol.* 50, 294-299
12. Bandlish, U., Prabhakar, B. R. and Virmani, (1991) U. Serum fucose levels in gynecological disorders including carcinoma cervix. *Journal Indian Med. Assoc.* 89, 250-251
13. Sen, U., Guha, S. and Chowdhury, J. R. (1985) A study of the prognostic role of serum fucose and fucosyl transferase in cancer of uterine cervix. *Acta Med. Okayama* 39, 125-30
14. Rao, V. R., Krishnamoorthy, L., Kumaraswamy, S. V. and Ramaswamy, G. (1998) Circulating levels in serum of total sialic acid, lipid associated sialic acid and fucose in precancerous lesions and cancer of the oral cavity. *Cancer Detect. Prev.* 22, 237-240
15. Fernandez-Rodriguez J., Paez de la Cadena, M., Martinez-Zorzano, V. S., and Rodriguez-Berrocal, F. J. (1997) Fucose levels in sera and in tumours of colorectal adenocarcinoma patients. *Cancer Lett.* 121, 147-153
16. Agroyannis, B., Dardoufas, C., Vitoratos, N., Tzanatos, H., Kouvaris, J., Damatapoulas, A., Koutsikos, D., Dalamangas, A. and Papavasiliou, C. (1994) Changes in serum transferrin and ceruloplasmin after radiation therapy in women with cervical or uterine carcinoma. *Clin. Exp. Obstet. Gynaecol.* 21, 24-27.
17. Arumanayagam, M., Wong F. W., Rogers, M. and Swaminathan, R. (1993) Serum ceruloplasmin, plasma copper concentration and copper to ceruloplasmin ratio in cervical carcinoma. *Gynaecol. Obstet. Invest.* 35, 175-178.
18. Chan, A., Wong, F. and Arumanayagam, M. (1993) Serum ultrafilterable copper, total copper and ceruloplasmin concentration in gynaecologic carcinomas. *Ann. Clin. Biochem.* 30,545-549.
19. Hansen, J. S., Iversen, J., Limhe, A. and Bog Hansen T. C. (1987) Acute phase reaction, heterogeneity and microheterogeneity of serum proteins as non-specific tumor markers in lung cancer. *Cancer* 60, 1630-1635.
20. Ros-Bullon, M. R., Sanchez-Pedreno, P. and Martinez-Liarte, J. H. (1999) Serum sialic acid in malignant melanoma patients: An ROC curve analysis. *Anticancer Res.* 19, 3619-22